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## Anti-Arrhythmic Hit to Lead Refinement in a Dish using Patient-Derived iPSCs

In the United States, almost one million individuals are hospitalized every year for cardiac arrhythmias, making arrhythmias one of the top causes of healthcare expenditures with a direct cost of almost $50 billion annually. Almost 300,000 individuals die of sudden arrhythmic death syndrome every year.

Medicinal chemists at the Human BioMolecular Research Institute (HBRI), in San Diego, CA, and stem cell biologists at Stanford University, in Stanford, CA, and cardiovascular pharmacologists at UCLA in Los Angeles, CA and Columbia University, in New York City, NY, respectively, reported on reengineered mexiletine compounds that showed improved potency and decreased toxicity in addressing ventricular cardiac arrhythmia.

Ventricular cardiac arrhythmia arises in individuals who have either acquired or congenital heart disease. Arrhythmias are very common in older adults but unfortunately, drugs to treat arrhythmias have liabilities. In addition, numerous investigational drugs have been withdrawn from the market because they induce QT prolongation and a potentially fatal ventricular tachycardia, a condition called Torsade de Pointes. Correct regulation of ion currents in cardiomyocytes is key for proper heart function. For normal heart cell function, sodium channels rapidly inactivate with depolarization. In depolarization of cardiomyocytes, sodium channels open briefly and allow influx of sodium. Correct regulation of ion currents in cardiomyocytes is key for proper heart function.

Cardiac action potential is initiated by the opening of the cardiac sodium channel conducting the large peak sodium current responsible for the action potential upstroke. In healthy individuals, the sodium channels inactivate with depolarization. However, Long QT syndrome type 3 (LQT3) patients have mutations in the sodium channel that impair channel inactivation and accelerate recovery from the inactivated state. Increased sodium channel current opposes repolarization and prolongs the action potential, thus prolonging the QT interval on the surface electrocardiogram and impairing function.

Mexiletine is a drug that inhibits the sodium current and shortens the QT interval in LQT3 patients and decreases their risk of developing ventricular tachycardia and ventricular fibrillation. Blockade of voltage-gated sodium channels that inhibits generation and propagation of action potentials can be anti-arrhythmic and prevent pathological firing in cardiac tissue. However, mexiletine has liabilities. For example, mexiletine inhibits the potassium channel. In addition, the FDA Approved Label states that severe liver injury and blood dyscrasias (i.e., leukopenia or agranulocytosis) and other adverse reactions including reversible gastrointestinal and nervous system problems have been reported after mexiletine treatment. Accordingly, in our studies, mexiletine analogs were designed to improve pharmaceutical properties, increase the potency and improve the therapeutic to toxicity ratio.

We tested the hypothesis that mexiletine that possesses liabilities (i.e., potassium channel inhibition and adverse reactions) could be re-engineered by dynamic medicinal chemistry and evaluated in normal and human patient induced pluripotent stem cell-derived cardiomyocytes to afford new drug candidates with greater efficacy and less toxicity.

The report showed that application of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to evaluate the proarrhythmic effects and safety of mexiletine analogs also helped to define chemical determinants responsible for anti-arrhthymia. In parallel, patient-derived hiPSC-CMs carrying a pathological LQT3 sodium channel mutation were used to quantify the efficacy of mexiletine and its analogues. Mexiletine analog beneficial effects were quantified for potency of shortening the action potential duration, fold-shortening of the action potential duration and a concentration that caused shortening of the action potential. The results led to identification of significantly more potent and selective sodium channel inhibitors with decreased proarrhythmic effects and much improved physicochemical properties. Compounds were shown to be effective anti-arrhythmic compounds in hiPSC-CM models of LQT3-associated arrhythmia.

This paper was featured in the *Journal of Medicinal Chemistry* in May, 2021.

*“The finding that “drug discovery in a dish” using a “disease in a dish” technology was successful is important because it showed that human drug development can be done efficiently using these approaches”* explains co-author Dr. Jorge Gomez-Galeno. *“By selectively blocking a sodium channel in human cardiomyocytes, and decreasing off-*

*target effects, new and safer drugs can be developed."* Given its potency, these new compounds may be of utility as antiarrhythmic drug candidates.

"*The new compounds may lead to treatment applications in a whole host of cardiovascular conditions that may prove efficacious in clinical trials*"… explains co-author Dr. John Cashman. "*As antiarrhythmic drug candidate drug development progresses, we expect the new analogs to be less toxic than current therapeutics for arrhythmia in* congenital heart disease*, and patients will benefit from improved safety, less side effects and possibly with significant cost-savings."*

**Summary**

Chemists and biologists at HBRI, Stanford University, UCLA, and Columbia University are among the first group to do dynamic medicinal chemistry of small molecules and use a high throughput assay employing drug development in a dish and disease in a dish technology to obtain pharmacologically active compounds of use in cardiovascular disease. New mexiletine analog compounds selectively blocked sodium channels. To our knowledge, this is one of the first reports to use human iPSC-derived cardiomyocytes from normal and diseased individuals to re-engineer a drug with deficiencies. Revealing links between drug development and selective human cardiomyocyte sodium channel inhibition might be fundamentally important and clinically relevant.

**Publication**

"Anti-Arrhythmic Hit to Lead Refinement in a Dish using Patient-Derived iPSC Cardiomyocytes. John R. Cashman, Daniel Ryan, Wesley L. McKeithan, Karl Okolotowicz, Jorge Gomez-Galeno, Mark Johnson, Kevin J. Sampson, Robert S. Kass, Arash Pezhouman, Hrayr S. Karagueuzian, and Mark Mercola. *Journal of Medicinal Chemistry*. https://doi.org/10.1021/acs.jmedchem.0c01545.

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**About Human BioMolecular Research Institute**

The Human BioMolecular Research Institute is a non-profit research institute conducting basic research focused on unlocking biological and chemical principles related to

diseases of the human brain, cardiovascular disease and cancer. The Institute conducts fundamental studies of central nervous system disorders, heart disease and cancer including stem cell approaches and translates findings into new drug development to address human illness. In addition, the institute promotes scientific learning through community service and public access by disseminating information and sharing research with collaborators, colleagues and the public. For more information, visit www.HBRI.org.

**About Stanford University, Stanford, CA**

Stanford University is a leading teaching and research institution. The University is organized around traditional schools consisting of academic departments at the undergraduate and graduate level and professional schools that focus on graduate programs in Law, Medicine, Engineering, and Business. It is recognized for exceptionally high achievement in research, clinical care, education and community outreach and partnerships. For more information, visit www.Stanford.edu.

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